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Pharmacokinetics and biliary excretion of rose bengal in rats with acute and chronic renal failure

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The effects of glycerol-induced acute renal failure (ARF) and surgically induced chronic renal failure (CRF) on the pharmacokinetics and biliary excretion of rose bengal have been examined in the rat. Both the pharmacokinetics and biliary excretion of rose bengal were unaltered in either ARF or CRF. The latter results in CRF contrast with those of Tse et al (1976, Int. J. Nucl. Med. Biol. 3: 134–137) who reported decreased removal of the dye from blood and reduced biliary excretion. In addition, rose bengal behaves differently from bromosulphophthalein and indocyanine green whose hepatic uptake and initial biliary excretion are known to be decreased in ARF. The results suggest that rose bengal may have a hepato-biliary transport route which differs from that of bromosulphophthalein and indocyanine green, and the findings also emphasize the selective nature of altered organic anion uptake by the liver in ARF.

Anionic dyes such as bromosulphophthalein, its analogue dibromosulphopthalein, indocyanine green and rose bengal have proved to be useful in the assessment of the effects of disease and xenobiotics on hepatic excretory function. This is because the liver is predominantly responsible for their removal from the body (Klaassen & Watkins 1984). Recent work has shown that the hepatic uptake and initial biliary excretion of bromosulphophthalein, dibromosulphophthalein and indocyanine green were decreased in rats with acute renal failure (ARF) (Bowmer et al 1982, 1986; Bowmer & Yates 1984). In addition, Tse et al (1976) found that the disappearance of rose bengal from blood and its biliary excretion were both reduced in rats with chronic renal failure (CRF). Thus it would appear that the way the liver handles these four dyes is altered to a similar degree in renal failure.

In rats with surgically induced CRF, Tse et al (1976) found a reduction of about 38% in the percentage dose of rose bengal excreted in bile after 1 h. By contrast, our work with bromosulphophthalein, dibromosulphophthalein and indocyanine green in rats with glycerol-induced ARF only demonstrated decreases in biliary excretion rates during the first 10 min after the administration of dye, with no significant change in the percentage dose excreted into bile (Bowmer et al 1982, 1986; Bowmer & Yates 1984). Why there should be such a contrast between the biliary excretion of rose bengal in CRF and the biliary excretion of other cholephilic dyes in ARF is not clear, but it may be related to the different models of renal failure used in these studies. Consequently, we have studied the pharmacokinetics and biliary excretion of rose bengal in rats with glycerol-induced ARF and compared the results with those obtained in rats with surgically induced CRF.

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Materials and methods

Chemicals. Rose bengal was purchased from Serva Feinbiochemica (Heidelberg). Reagents for the assay of plasma urea were obtained from Sigma Chemical Co. All other reagents were of analytical grade and were available commercially.

Induction of ARF. ARF was induced in male Wistar albino rats (270–350 g) by an i.m. injection of 50% (v/v) glycerol in sterile 0·9% NaCl (saline), 10 mL kg^{-1} body weight (Thiel et al 1967). Control rats received an injection of saline (10 mL kg^{-1}) and both groups of rats were studied 48 h after the injection of glycerol or saline.

Induction of CRF. Male Wistar albino rats (90–125 g) were anaesthetized with ether and underwent partial nephrectomy, whereby two thirds of the right kidney was removed at the first operation and the left kidney was removed one week later (Young et al 1973). Sham operations, where the capsule was removed from the right kidney and the left kidney was exposed, were performed on a control group of rats. The animals were studied 28 days after the completion of surgery.

Experimental protocol. Rats were anaesthetized with pentobarbitone (60 mg kg^{-1}) and cannulae inserted into the trachea, left jugular vein, right carotid artery and common bile duct. Rose bengal was injected via the jugular vein as an aqueous solution (10 mg kg^{-1}, 10 mg mL^{-1}). Heparinized blood samples (0·1 mL) were removed at suitable intervals over 60 min and bile was collected over 5 or 10 min periods for 1 h. Aliquots (50 μL) of plasma and bile were diluted with an appropriate volume of 0·1 M NaOH and the optical density at 553 nm was measured.

Pharmacokinetic calculations. Concentration-time curves were fitted to a biexponential equation by derivative-free, non-linear least squares regression analysis using the BMDP statistical software package (Ralston 1983). Data were analysed using a two compartment model, with elimination of rose bengal from the peripheral compartment (Richards et al 1959). In this model k_{12} is the first order rate constant for transport of dye from plasma into liver, k_{21} the rate

constant of efflux of dye from the liver back into plasma and k_{23} the rate constant for excretion into the bile. These rate constants together with the plasma clearance (Clp) and the apparent volume of distribution at steady-state (Vd_{ss}) were calculated as described previously (Bowmer et al 1982).

Values are expressed as mean ± s.e. mean and statistical comparisons were made using the non-paired Student's *t*-test.

Results

Mean plasma urea concentrations in saline-injected and sham-operated control rats were 36 ± 3 (n = 9) and 50 ± 2 (n = 7) mg dL^{-1}, respectively. In rats injected with glycerol, urea concentrations increased to 263 ± 61 (n = 8) mg dL^{-1} and in rats which had undergone partial nephrectomy urea levels were 126 ± 7 (n = 7) mg dL^{-1}. There were no significant differences in either mean body weight or wet liver weight between rats with renal failure and their respective control group.

Fig. 1 depicts the plasma concentration-time curves obtained after i.v. injection of rose bengal (10 mg kg^{-1}) to control rats and rats with ARF. In both groups of rats rose bengal appeared to be removed in a biexponential manner. There were no significant differences in mean plasma concentrations between these two groups of rats. Consequently, the half-times for the initial disappearance phase ($t_{0.5\alpha}$), terminal elimination phase ($t_{0.5\beta}$) and the other kinetic parameters obtained for rats with ARF were unchanged when compared with control values (Table 1). The biliary excretion rate-time profile for rose bengal is shown in Fig. 2. There were no significant differences in biliary excretion rates at any time interval between these groups of animals. Furthermore, the percentage of rose bengal recovered in bile after 1 h was similar between control rats (45 ± 2·5; n = 5) and rats with ARF (46 ± 2·6; n = 5). The mean bile flow rate over 1 h in control rats (7·8 ± 0·43 μL min^{-1}/100 g; n = 5) was not significantly different from that in rats with ARF (6·8 ± 0·32 μL min^{-1}/100 g; (n = 5).

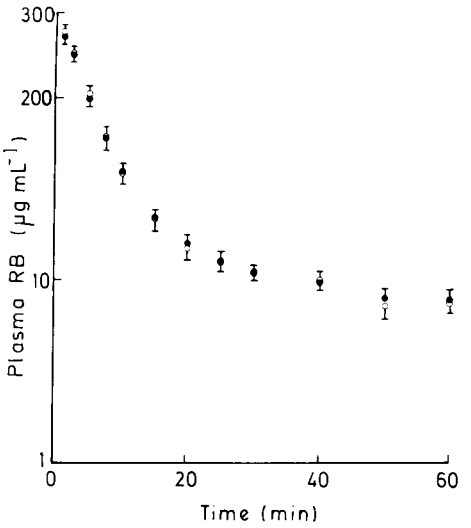

FIG. 1. Plasma concentrations of rose bengal (RB) after intravenous administration (10 mg kg^{-1}) in control rats (○) and rats with glycerol-induced acute renal failure (●). Values are mean ± s.e. mean (n = 9 and 8, respectively).

The disappearance of rose bengal from the plasma of both sham-operated rats and rats with CRF was almost identical to that seen previously in the acute experiments. The decay curves from sham-operated and CRF groups were superimposable and consequently no significant differences were observed in the pharmacokinetic parameters from each of the two groups (Table 1). Mean biliary excretion rates over 1 h were not significantly altered in rats with CRF and the percentage dose excreted in bile over this period (49 ± 1·8; n = 7) was similar to that found in the sham-operated group (46 ± 1·2; n = 7). Again mean bile flow rate was not statistically different between the two groups of rats. In controls flow rate was 6·3 ± 0·36 μL min^{-1}/100 g (n = 7) whereas in rats with CRF it was 6·7 ± 0·33 μL min^{-1}/100 g (n = 7).

Table 1. Effect of glycerol-induced acute renal failure and surgically induced chronic renal failure on the pharmacokinetics of rose bengal (10 mg kg^{-1} i.v.).

Pharmacokinetic parameter	Saline-injected control rats (n = 9)	Glycerol-injected rats (n = 8)	Sham-operated control rats (n = 7)	Rats with partial nephrectomy (n = 7)
$t_{0.5\alpha}$ (min)	3·0 ± 0·1†	3·2 ± 0·3	2·9 ± 0·1	2·8 ± 0·1
$t_{0.5\beta}$ (min)	58 ± 11	79 ± 10	44 ± 8	57 ± 8
k_{12} (min^{-1})	0·23 ± 0·01	0·22 ± 0·02	0·22 ± 0·02	0·24 ± 0·01
k_{21} (min^{-1})	0·014 ± 0·002	0·013 ± 0·001	0·012 ± 0·001	0·014 ± 0·002
k_{23} (min^{-1})	0·015 ± 0·002	0·011 ± 0·002	0·017 ± 0·003	0·014 ± 0·002
Cl_p (mL min^{-1}/100 g body weight)	0·40 ± 0·03	0·38 ± 0·03	0·40 ± 0·02	0·40 ± 0·02
Vd_{ss} (mL/100 g body weight)	35 ± 4	48 ± 5	26 ± 3	33 ± 3

† Values are mean ± s.e. mean.

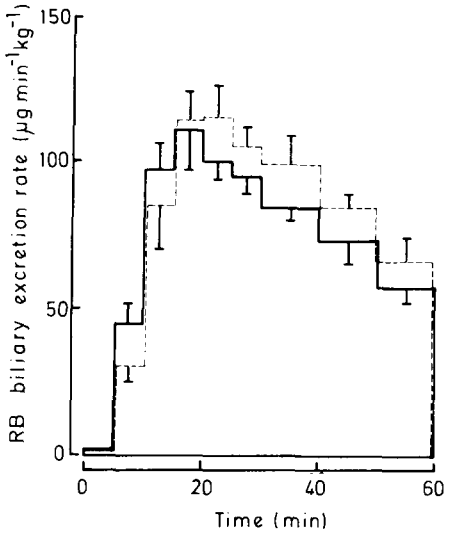

FIG. 2. Biliary excretion profile of rose bengal (RB, 10 mg kg^{-1}, i.v.) in control rats (unbroken line) and rats with glycerol-induced acute renal failure (broken line). Values are mean ± s.e. mean (n = 5).

Discussion

In the present study none of the pharmacokinetic values showed any significant deviation from corresponding control values after the induction of either ARF or CRF. Moreover, in both groups of rats with renal failure, the values of $t_{0.5\alpha}$, k_{12} and Clp, which together constitute a measure of the hepatic uptake of rose bengal, were almost identical to those obtained in the respective control groups. Biliary excretion rate, bile flow rate and percentage dose recovered in bile were all unaltered in rats with either glycerol-induced ARF or surgically induced CRF. Thus the elimination of rose bengal into bile seems to be unchanged in these models of experimental renal failure. Together the results of these experiments suggest that the hepatic handling of rose bengal is unaltered in rats with renal failure.

The results for rats with CRF clearly contrast with those obtained by Tse et al (1976) who found a 22% decrease in α, the rate constant of the initial disappearance phase, and a 38% decrement in the biliary excretion of rose bengal in rats with surgically induced CRF. There are, however, a number of problems with the work of Tse et al (1976) which make comparison with the present study difficult. For example, no mention was made of either bile flow rate or biliary excretion rate. Furthermore, α was given without units and its value was about sixty times less than those that can be calculated from the half-times of the initial disappearance phase ($t_{0.5\alpha}$) given in Table 1. The values of $t_{0.5\alpha}$ listed in Table 1 are in good agreement with that found by Klaassen (1976) using the same dose of dye.

Why we were unable to obtain results similar to those of Tse et al (1976) is not clear, but it may be related to differences in dose, to a lack of suitable controls or to the duration of CRF. Tse et al (1976) used ^{125}I-labelled dye and a dose of 0·1 mg per rat whereas about 3 mg per animal (10 mg kg^{-1}) was used in the present study. However, previous work with indocyanine green showed that altered hepatic uptake was more apparent at a relatively high dose (7·5 mg kg^{-1}) than at lower doses (4 and 1 mg kg^{-1}) (Yates et al 1983a). Tse et al (1976) did not subject their control animals to sham surgical procedures, yet it is known that surgery can alter the removal of cholephilic dyes such as indocyanine green from plasma (Bowmer et al 1982). The rats studied by Tse et al (1976) were used 60 days after the final surgical procedure. This should allow adequate recovery from any acute effects of surgery on liver function. In addition, Table 1 shows that there were no significant differences in the kinetics of rose bengal between saline-injected and sham-operated rats which suggests that surgery itself had no effect on the elimination of rose bengal. In our studies, CRF was allowed to develop over 28 days. This period may not have been sufficient for liver function to be substantially altered, but we have previously noted decreased hepatic uptake of indocyanine green at this stage in CRF (Yates et al 1983b).

Rose bengal is an organic anion of similar molecular weight and structure to dyes such as bromo- and dibromosulphophthalein and like these latter compounds it is highly bound to plasma proteins (Meurman 1960) as well as being rapidly removed from plasma and excreted into bile with little extravascular distribution (Meurman 1960). But there is evidence that rose bengal may be handled by the liver in a manner different from the two phthalein dyes. Schwenk et al (1976) showed that rose bengal did not inhibit the uptake of bromosulphophthalein into rat isolated hepatocytes. Furthermore, Mahu et al (1977) found that the transport of rose bengal into bile differed from that of bromo- and dibromosulphophthalein under experimental conditions of fast, phenobarbitone pretreatment and bile salt infusion. These workers concluded that rose bengal does not share the same liver-to-bile excretory pathway as phthalein dyes such as bromosulphophthalein.

The kinetics and biliary excretion of rose bengal were unaltered in rats with ARF and this contrasts with the decreased hepatic uptake and delayed biliary excretion noted previously with bromosulphophthalein, dibromosulphophthalein and indocyanine green in this model of ARF (Bowmer et al 1982, 1986; Bowmer & Yates 1984). This lack of effect is interesting in two respects. Firstly, it lends some support to the conclusion reached by Mahu et al (1977) that rose bengal may have a hepato-biliary transport route which differs from that utilized by bromo- and dibromosulphophthalein. Secondly, we have suggested that the impairment of hepatic uptake and excretory function in ARF is confined to the pathway responsible for the excretion of bromosulphophthalein, dibromosulphophthalein and

indocyanine green (Bowmer & Yates 1984; Silberstein et al 1986). This suggestion was based on observations that whereas the hepatic handling of these dyes was altered in ARF, no change in hepatic uptake or biliary excretion of the neutral glycoside ouabain, the organic cation *N*-acetylprocainamide ethobromide and the bile acid taurocholic acid could be detected (Bowmer & Yates 1984; Silberstein et al 1986). Thus the results obtained for rose bengal further highlight the selective nature of this defect in liver function in ARF.

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